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A Comparison of the Recovery of Injured *Escherichia coli* O157:H7 in Five Commercial Formulations of Buffered Peptone W
Using Indirect Impediometry.

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BACKGROUND. A Northern Ireland survey of 780 beef carcasses (based on excised meat samples) found no *E. coli* O157:H7 although it was detected in faeces submitted to a veterinary investigation centre at a low rate (Ball and Madden, 1997). Since infection with *E. coli* O157:H7 can cause severe illness, and even death, it is essential that methods used for the isolation of *E.* O157:H7 from meats and other food stuffs are as efficient as possible. Optimising culture media and conditions by conventiona methodologies is a slow and expensive process due to the considerable amount of staff-hours involved in enumerating bacteria prepare growth curves for comparison. However Donaghy and Madden (1993) showed that indirect impediometry (Owens et a 1989) can be used to rapidly estimate, and compare, the growth of salmonellas in broths. This work also showed that predictio isolation efficiency based on impedance were upheld when naturally contaminated meat samples were studied. Thus the speed impediometry, and simplicity of studies with pure cultures, could allow a rapid comparison of commercial media to determine would be best to be applied to the isolation of *E. coli* O157:H7 from meats and other food samples.

OBJECTIVES. To enhance the isolation of *E. coli* O157:H7 from foods samples of the foodstuff are normally incubated in a n selective broth allowing the bacteria to repair any damage, and then to proliferate, subsequent to plating on a selective medium increases the probability of the organism being detected. Buffered peptone water (BPW) has been used as a basal medium for t purpose (Chapman et al, 1993) and incubated at 37°C. The aim of these studies was firstly to investigate the effect of four stres 12 isolates of *E. coli* O157:H7 in order to select three for the subsequent comparison of rates of recovery of injured cells in fiv different commercial formulations of BPW. Indirect impediometry (Owens et al, 1989) was chosen to compare the growth rate injured cells. Thus the best recovery medium could be selected for application in routine studies

METHODS. A Rapid Automatic Bacterial Impedance Technique (RABIT) system (Don Whitley Scientific, Shipley, GB) was for all impediometry. *E. coli* O157:H7 isolates utilised (12) were obtained from patients, animals and foodstuffs, and one type from the national Collection of Type Cultures, London (NCTC 12079). Cultures were maintained on Protect beads (Technical Services Consultants Ltd, Lancaster, GB) at -80°C. Prior to use cultures were grown overnight in nutrient broth (100ml) then harvested by centrifugation (1700g (av.), 10min). The pellet was resuspended in 10ml phosphate buffered saline (PBS) for sub use. Three stress regimes were used; i) heating in a water bath at 52°C for 30 minutes, ii) freezing at -80°C for 15min followed thawing at 25°C for 50min, this cycle being repeated four times, iii) resuspending the pellet noted above in PBS supplemented 25% (w/v) NaCl and held at 30°C for 2 hours , iv) resuspending the pellet noted above in PBS supplemented with 30% (w/v) N and held at 30°C for 2 hours. Damage was estimated by plating simultaneously on tryptone soy agar and sorbitol MacConkey supplemented with cefixime and potassium tellurite (CT-SMAC agar) (24h, 37°C) to determine total counts and resistant cells respectively. Indirect impediometry used RABIT cells to which equal volumes of molten 2% (w/v) agar (Oxoid No.1) and 0.7 KOH solution were added. The cells were stoppered and left overnight to stabilise. Buffered peptone water broths for study w prepared to the manufacturers specifications then dispensed (4.5ml) into sterile glass tubes (12x75mm). The inoculation volun 0.1ml and the detection criterion for the RABIT was -10μS, with impedance values logged every 6 min for 24h. Samples were analysed in duplicate with four decimal dilutions being inoculated from each test strain; 10^{-1} to 10^{-4} dilutions were used in all

RESULTS AND DISCUSSIONS. It was seen that the heat stress applied had the highest lethality whilst all of the 12 isolates most resistant to the freeze/thaw treatment, Fig.1. The salt stresses applied were intermediate in lethal effect and the higher lev salt, 30% (w/v) was adopted for the main investigation due to its greater lethality.

Typical impediometry results are shown in Fig. 2, and illustrate the time to detection (TTD) values obtained with one isolate on Lab M BPW following exposure to the heat and freeze/thaw treatments. The TTD value is the time taken for the inoculum grow to a pre-set level of activity, in this case the evolution of sufficient CO_2 to change the impedance of the KOH gel by the parameters noted above. Hence the TTD measures the time the inoculum takes to repair, pass through lag phase and then grow logarithmically until numbers are sufficiently high for the CO_2 evolved to be detected: usually from 10^6 to 10^7 cfu/ml. The experimental design yielded eight data points per isolate, per treatment and regression analysis was conducted to calculate the of TTD vs. dilution factor, i.e. the growth rate of the isolate in terms of the time taken for one log cycle of growth (a ten-fold in numbers). This growth rate was then calculated for all of the isolates and the medium providing the fastest growth determin

Manufacturer of BPW	Growth rate[1]		TTD[2] at 0 diln
Difco	2.09		6.51
Lab M	1.23		5.01
Mast	1.52		4.94
Merck	1.32		5.29
Oxoid	1.26		4.97

[1]Time taken, in hours, for 1 log cycle increase in numbers. [2]Time to detection (h).

Table 1. Mean growth rates and intercept values of 12 *E. coli* O157:H7 isolates injured by three different stresses, in five form of buffered peptone water (BPW), obtained by regression analysis.

However the lag phase of the cultures must also be considered since a long lag phase is undesirable in a recovery medium. This value cannot be directly obtained from the results but an indication of the relative length of the lag phase can be found by studying the intercept calculated from the regression analysis, in conjunction with the growth rate. The intercept is the predicted TTD at a dilution factor of 0 and will hence represent the time taken for lag phase to be completed, followed by exponential growth to the critical number of cells needed for the RABIT to detect the change of impedance. Since identical inocula are used media yielding identical growth rates can be ranked by comparing their intercept TTD values since the only cause of any difference must be the length of the lag phase.
Thus Table 1 indicates that Lab M BPW provides the best recovery and subsequent growth of injured *E. coli* O157:H7 of all the media studied.
Considering the other media it can be seen that the Difco medium is clearly the poorest giving the slowest growth found by a considerable margin. Growth on the Mast medium was also noticeably slower than on the best three media (Lab M, Oxoid, Merck).

CONCLUSIONS. The choice of formulation of buffered peptone water considerably affected the rate of recovery of 12 *E. coli* O157:H7 isolates injured by three different stress regimes. Based on previous studies (Donaghy and Madden, 1993) these results would be reflected in the application of these media to the recovery of injured *E. coli* O157:H7 from foodstuffs. Whilst the differences between the three best media are probably not of practical significance studies are in progress to compare the ability of best and worst media to recover *E. coli* O157:H7 from ground beef.

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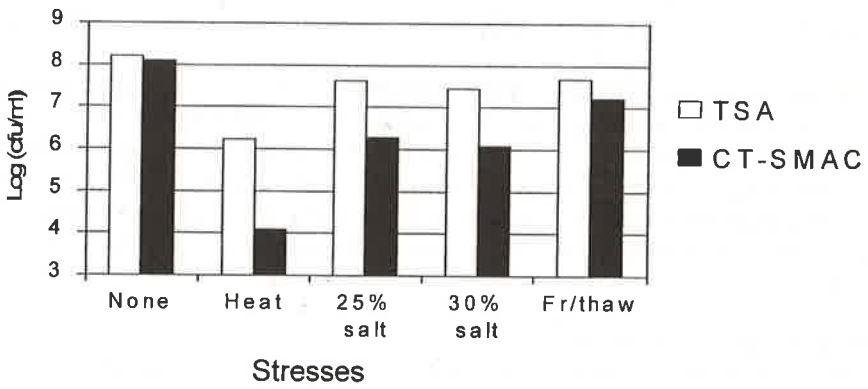

Figure 1.Effect of four stresses on recovery of 12 *E. coli* O157:H7 isolates on a selective medium, CT-SMAC and a non-selective medium, TSA.

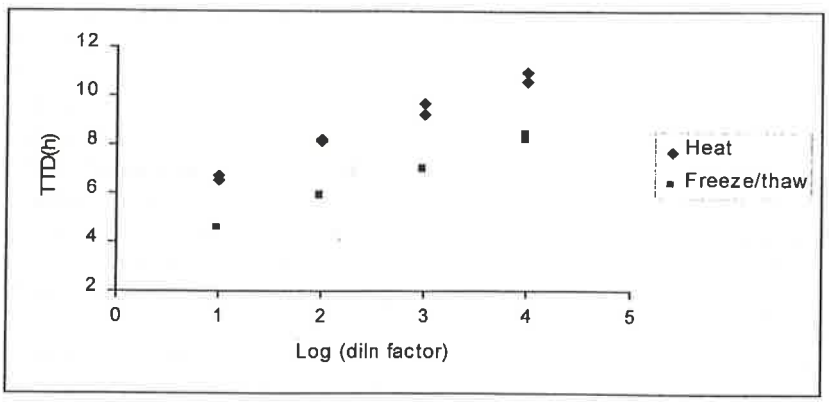

Figure 2. Time to detection (TTD) values obtained with one isolate subjected to two different stresses.